IDENTIFICATION OF PUTATIVE SHARK (SQUALUS ACANTHIAS) VITELLOGENIN

Lorelei E. Perez and Ian P. Callard.
Department of Biology, Boston University, 5 Cummington Street, Boston, MA 02215

Vitellogenin, a complex lipophosphoglycoprotein, is the main source of nutrients for embryos of non-mammalian, vertebrate species. The protein is synthesized in the liver, transported in the blood stream to the ovary where it is taken up via receptor-mediated endocytosis by the oocyte. Once in the ovary, it is cleaved into smaller yolk components and stored for embryonic use.

Vitellogenin has been isolated in many species, most notably Xenopus and chicken [Wallace, R.A. In: Dev Biol vol I, L. Browder, ed., Plenum, NY,1985]. However, little work has been done on the earliest gnathostomes, the elasmobranchs [Woodhead, P.M.J.,Gen Comp Endocrinol, 13:310,1969; Craik, J.C.A., Gen Comp Endocrinol, 35:455,1978]. The elasmobranchs are unique, being the earliest group of extant vertebrates to have evolved viviparity and the associated changes in vitellogenin production and development of placentae and placental analogs. We have recently described the isolation and purification of vitellogenin in the oviparous skate Raja erinacea [Perez,L.E. and Callard, I.P., in preparation, 1992a]. Here, we describe the identification of putative vitellogenin in the viviparous dogfish, Squalus acanthias. To our knowledge, this is the first report on the identification of vitellogenin in a viviparous elasmobranch.

A pregnant female shark (stage C) was bled from the caudal vein into heparinized tubes containing 1 mM PMSF (phenyl-methyl sulfonyl fluoride) and 1 mM leupeptin to inhibit protease activity. Plasma was collected after centrifugation and vitellogenin precipitated with the addition of 20 mM EDTA and 0.5 M MgCl2. All procedures were performed at 4 ^{o}C. The mixture was incubated overnight, and centrifuged for 15 minutes at 2500 x g. The pellet was resuspended in 3 ml 1 M NaCl/50 mM Tris-HCl, pH=7.5 and centrifuged to remove debris. The supernatant was re-precipitated with the addition of 8 volumes of water containing 1 mM PMSF/1 mM leupeptin. The mixture was incubated overnight and the precipitate centrifuged at 2500 x g for 15 minutes. The pellet was resuspended in 3 ml 1M NaCl/50 mM Tris-HCl, pH=7.5, de-salted and concentrated by centrifugation on Centricon-100 (Amicon) filtration tubes, lyophillized and stored at -70 ^{o}C. Samples were run on 0.75 mm, 6% SDS-PAGE using the buffer system of Laemmli [Nature, 227:680, 1970]. Gels were stained in 0.2% Coomassie blue for visualization of proteins. Duplicate gels were transferred onto nitrocellulose membranes and probed with rabbit anti-skate vitellogenin antibody [Perez L.E. and Callard, I.P., in preparation, 1992b].

The addition of $MgCl_2$/EDTA precipitated a wide spectrum of proteins as seen on polyacrylamide gels. However, 3 prominent high molecular weight bands at 241, 225, and 207 kD respectively appeared in female plasma. No proteins were precipitated by MgCl2/EDTA from male plasma (data not shown). Western blot analysis of female dogfish precipitates with anti-skate vitellogenin showed weak, binding with only the high molecular weight triplet proteins (data not shown). Interestingly, female skate plasma probed with rabbit anti-skate vitellogenin antibody exhibits strong specific binding with only one protein band at 205 kD [Perez L.E. and Callard, I.P., in preparation, 1992b]. Problems with degradation of precipitated proteins occurred thoughout the procedure, even in the presence of PMSF and leupeptin, which is common when working with this protein (see Wallace, 1985, ibid). This may explain the presence of smaller molecular weight proteins near the dye front in the gel, although we cannot rule out the possibility of non-selective precipitation. Further purification steps utilizing DEAE-cellulose chromatography resulted in complete degradation of the triplet protein.

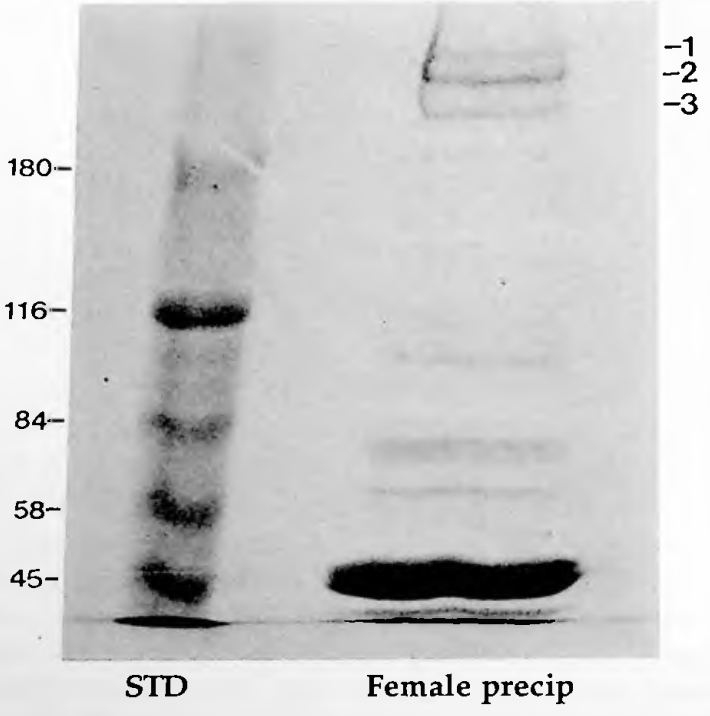

FIGURE 1 - $MgCl_2$/EDTA precipitation of female dogfish (Squalus acanthias) plasma. A triplet of high molecular weight proteins (241, 225, 207) kD, which weakly binds to anti-skate vitellogenin antibody, is precipitated with this technique. STD = standard; Female precip = female dogfish $MgCl_2$/EDTA precipitate.

Taken together, the data suggest that the triplet protein isolated from female dogfish plasma has similar biochemical characteristics to vitellogenin, being selectively precipitated with $MgCl_2$/EDTA. This technique has previously been used to precipitate Xenopus, Rana, Gallus, and a variety of other vertebrate vitellogenins [Wiley et al, Anal. Biochem., 97:48,1980; Carnevali and Polzonetti-Magni, J. Exper. Zool., 259:18,1991]. In addition, anti-skate vitellogenin antibody weakly, but specifically, recognizes the triplet protein in Western blots. In the appearance of a triplet of proteins, the dogfish differs from the oviparous Raja erinacea, where only one vitellogenin subunit could be detected. This suggests that vitellogenin has structurally evolved within the elasmobranch group. These differences may relate to the evolution of viviparity and associated changes in vitellogenin genes.

Supported by NIH 1 RO1 RR 06633-01 to IPC and an NSF pre-doctoral fellowship to LEP.